AD-A258 020

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	01 Sep 91 TO 31 Aug 92

4. TITLE AND SUBTITLE

BIODEGRADATION OF JET FUEL-4 (JP-4) IN SEQUENCING BATCH REACTORS

5. FUNDING NUMBERS

G AFOSR-91-0404
PE 61102F
PR 2312
TA A4

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

AFOSR-TR- 92-0994

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

AFOSR/NL
Building 410
Bolling AFB DC 20332/6448
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10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Several bacterial consortia were evaluated for use in the development of a sequencing batch reactor (SBR) for bioremediation of water contaminated with Jet Fuel-4. (JP-4). All of the bacterial systems tested had some ability to degrade components of JP-4. However, because of its ability to degrade rapidly components of JP-4 and because of its rapid oxygen utilization rates, a bacterial consortium from a soil contaminated with gasoline was selected for further study and was used in the development of an SBR to treat contaminated water. The bench scale SBR developed had a three liter capacity and a 1.5 liter head space. The atmosphere in the head space was changed only during the fill and draw portion of the reactor cycle. At other times, the system was closed and the only oxygen available was that dissolved in the water being treated or in the atmosphere of the headspace. Calculations revealed and experimental data confirmed that this amount of oxygen was sufficient to support extensive bioremediation of water contaminated with JP-4. Results demonstrated that this SBR was consistently able to bioremediate water contaminated with JP-4. During 12 hr cycle times the amount of JP-4 present (30 - 39 mg/L) was always reduced to less than 50 ug/L and usually to below 20 ug/L was always reduced to less than 50 ug/L and usually to below 20 ug/L and met the

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
(U)	(U)	(U)	(U)

regulatory limits for total petroleum hydrocarbons mamdated by all but the strictest state regulatory agencies. This SBR has been in continuous operation for over 135 days. Initial characterization of the bacterial consortium used in this reactor revealed the presence of Pseudomonas luteola and Aeromonas sp., both of which were able to grow on JP-4 fumes as a sole carbon source.

Report UND-AFOSR-1-91/92

Biodegradation of Jet Fuel 4 in Sequencing Batch Reactors

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22 July 1992

Annual Technical Report September 1, 1991 to August 31, 1992

Prepared for:

Air Force Office of Scientific Research
Bolling Air Force Base, D.C. 20332-6448

Principal Investigator: Dr. John A. Bumpus

Project Personnel:

1. Mr. M. Keith Wyatt (10-15-92 to 4-15-92)
2. Ms. Sue Han (2-1-92 to present)
3. Dr. Robert Irvine (10-15-92 to present)
4. Mr. Paul Y, Yocum (1-15-92 to present)
5. Mr. Daniel Vaughn (6-15-92 to present)
6. Ms. Laura Perron (5-1-92 to 6-16-92)

The Specific Objectives of This Proposal Are:

1. To assess the ability of *C. resinae, P. chrysosporium* and selected bacterial consortia to degrade individual chemical components of JP-4.

2. To develop a sequencing batch reactor that utilizes *C. resinae* to degrade chemical components of JP-4 in contaminated water.

3. To develop a sequencing batch reactor that utilizes *P. chrysosporium* to degrade chemical components of JP-4 in contaminated water.

4. To develop a sequencing batch reactor that utilizes a bacterial consortium to degrade chemical components of JP-4 in contaminated water.

Status Report:

The overall objective of this research was to develop sequencing batch reactors (SBRs) that are able to remediate water contaminated with JP-4. Furthermore, it was proposed that SBRs could be designed which lower the amount of total petroleum hydrocarbons in water to levels that are below regulatory concern.

To date, we have been successful in developing a bench scale SBR using a bacterial consortium that is rather effective in the bioremediation of water contaminated with JP-4. The bench scale SBR developed had a three liter capacity and a 1.5 liter head space. Results demonstrated that this SBR was consistently able to bioremediate water contaminated with JP-4. During 12 hr cycle times the amount of JP-4 present (30 - 39 mg/L) was reduced to less that 50 ug/L, meeting the regulatory limits mandated by all but the strictest state regulatory agencies. This reactor has been in continuous operation for more that 135 days.

It was also our objective to evaluate two fungal systems and a commercially available bacterial consortium for use in SBRs. This objective has been expanded to include the evaluation of several other bacterial consortia and

has led to the discovery of the effectiveness of the bacterial consortium used in the aboved described SBR. This particular consortium was derived from soil contaminated with gasoline at the Los Angeles Air Force Base. The study of the effectiveness of the two fungal systems (*P. chrysosporium* and and *C. resinae*) is still in progress. Initial studies, however, suggest that rates of JP-4 degradation in fungal systems may be considerably slower than those observed in bacterial systems.

Significant Achievements:

1. An effective piltot scale SBR has been designed and constructed that is able to remediate water contaminated with JP-4 to levels that are below regulatory concern for most states.

2 The design of the SBR used in this study is such that loss of JP-4 by air stripping is negligible (i.e., approximately 1%).

3. The bacterial consortium used in the SBR mentioned above has been partially characterized. *Pseudomonas luteola* and *Aeromonas sp.* were identified. Both of these bacteria were able to grow on JP-4 fumes as their sole carbon source.

Publications:

Bumpus, J.A., Han, X., Irvine, R.L. and Yocum, P. (1992) *Biodegradation* of Jet Fuel-4 in a Sequencing Batch Reactor. (This manuscript will be submitted to Water Research, Water Environment Research or another appropriate peer reviewed journal that publishes research centering on water remediation.)

Interactions.

The above mentioned manuscript will be presented as a poster at the Hazardous Waste Conference that is to be hosted by the Center for Bioengineering and Pollution Control, University of Notre Dame and Miles, Inc. This conference will be held August 31, 1992 - September 4, 1992.

In January, 1992, I visited Grissom Air Force Base, Grissom, IN. At that time Lt. Col. Harold Vice gave me 2 gallons of JP-4. I also visited with other officers and enlisted personnel who kindly answered several questions concerning the properties of JP-4 and its proper storage.

Mr. Paul Yocum is a graduate student in Dr. Robert L. Irvine's research group and is a doctoral candidate in the Department of Civil Engineering and Geological Sciences. Much of the research described in this report was conducted by Mr. Yocum and will comprise part of his Ph.D. dissertation. He is expected to complete the requirements for the Ph.D. degree in January 1993. Another graduate student supported by this grant, Mr. Daniel Vaughn, joined the Center for Bioengineering and Pollution Control in June 1992. Mr. Vaughn is in

the process of developing a fixed film sequencing batch reactor for the bioremediation of water contaminated with JP-4.

New Discoveries, inventions, patent disclosures and specific applications.

1. A sequencing batch reactor has been designed and used in bench scale piltot studies. It is effective for the bioremediation of water contaminated with JP-4. Given the similarity of JP-4 with other fuels, it is likely that this system could also remediate water contaminated by JP-5, gasoline, kerosine and diesel fuel.

2. Bioventing is an effective method of treating soil that is contaminated with JP-4 and other volatile solvents. However, by its very nature, bioventing produces a highly contaminated off-gas which must also be treated. Catalytic oxidation is an effective, but expensive, method used to treat off-gases from bioventing operations. As noted above, two of the bacteria isolated from the Los Angeles Air Force Base grow on JP-4 fumes as their sole carbon source. Although it is not a specific objective of our current research, we suggest that these bacteria may be useful in developing cost-effective biofilters to remediate off-gases from bioventing operations.

Technical Summary.

The following technical summary is an overview of our AFOSR sponsored research completed to date.

Biodegradation of Jet Fuel-4 in a Sequencing Batch Reactor:

A Technical Summary of the First Year's Research
Air Force Office of Scientific Research
AFOSR-92-03-00015

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Abstract

Several bacterial consortia were evaluated for use in the development of a sequencing batch reactor (SBR) for bioremediation of water contaminated with Jet Fuel-4. (JP-4). All of the bacterial systems tested had some ability to degrade components of JP-4. However, because of its ability to degrade rapidly components of JP-4 and because of its rapid oxygen utilization rates, a bacterial consortium from a soil contaminated with gasoline was selected for further study and was used in the development of an SBR to treat contaminated water. The bench scale SBR developed had a three liter capacity and a 1.5 liter head space. The atmosphere in the head space was changed only during the fill and draw portion of the reactor cycle. At other times, the system was closed and the only oxygen available was that dissolved in the water being treated or in the atmosphere of the headspace. Calculations revealed and experimental data confirmed that this amount of oxygen was sufficient to support extensive bioremediation of water contaminated with JP-4. Results demonstrated that this SBR was consistently able to bioremediate water contaminated with JP-4. During 12 hr cycle times the amount of JP-4 present (30 - 39 mg/L) was always reduced to less than 50 ug/L and usually to below 20 ug/L and met the regulatory limits for total petroleum hydrocarbons mandated by all but the strictest state regulatory agencies. This SBR has been in continuous operation for over 135 days. Initial characterization of the bacterial consortium used in this reactor revealed the presence of *Pseudomonas luteola* and *Aeromonas sp.*, both of which were able to grow on JP-4 fumes as a sole carbon source.

Introduction

Jet Fuel-4 is a major fuel used by the United States Air Force. As a consequence of its extensive use, there are many sites that have been contaminated by accidental spills of JP-4. Although limited toxicological data are available about JP-4 *per se*, individual components such as benzene are known carcinogens and others are CNS depressants. Exposure of humans to petroleum distillates similar to JP-4 is known to cause headache, CNS depression, nausea, respiratory tract irritation and mental confusion. A review of the toxicology of JP-4 components is provided in the *The Installation Restoration Program Toxicology Guide* (1).

In addition to soil remediation, ground water contaminated by JP-4 is a matter of concern. For the bioremediation of ground water, two basic approaches exist; those which focus on *in situ* treatment and those in which a pump and treat approach is used. Due to ever increasing regulations regarding air pollution, pump and treat strategies must take care to minimize loss of petroleum hydrocarbons by air stripping. In the present investigation, we have developed a sequencing batch reactor for the bioremediation of water contaminated with JP-4. A unique feature in the design of this reactor is that oxygen is supplied to the system only during the fill and draw portion of the cycle to minimize air stripping.

Methods and Materials

Microorganisms. Two bacterial consortia were obtained from soils, one contaminated with gasoline and one with diesel fuel. The soil contaminated with gasoline was from the Los Angeles Air Force Base (Fort MacArthur Annex), Los Angeles, CA. Contamination of this soil was caused by leaking underground gasoline storage tanks. This soil has been contaminated since approximately 1945. The soil sample studied was taken from 30 ft below the surface in the capillary fringe. The soil contaminated with diesel fuel was from the Sandia National Laboratories, Livermore, CA. In 1978, 60,000 gallons of diesel fuel was spilled and percolated into the vadose zone to a soil depth of 106 ft. The soil sample studied was from a depth of 60.5 ft. A third bacterial consortium was obtained from the local municipal waste treatment facility (South Bend, Indiana) and a fourth (Formulation L-104) was a gift from the Solmar Corporation (Orange, CA).

Reactor Design. The SBR used in this investigation was based on the general description of SBR design and operation described by Irvine and Ketchum (2).

Jet Fuel-4. JP-4 was obtained from Grissom Air Force Base, Grissom, Indiana and was stored in grounded 1 gallon metal containers at room temperature until used.

Gas chromatography. TPH (Total petroleum hydrocarbons) and BTEX (benzene, toluene, ethyl benzene and xylenes) were extracted from treated or untreated water using a Tekmar 4000 heated purge and trap apparatus according to conditions specified in EPA Methods 8015 and 5030. The trapped

compounds were analyzed by gas chromatography using a Varian Model 3700 gas chromatograph equipped with an auto injector, a flame ionization detector (FID), a PE Nelson data acquisition system and a Supelco SPB-1 glass capillary column (60 m x 0.75 mm I.D.with a 1 um film thickness). Compounds were eluted isothermally at 40°C for 4 min. followed by a temperature gradient from 40°C to 210°C at 4°C/min. Helium was used as the carrier gas. TPH were quantitated by comparing area counts of unknowns with the total area counts of known JP-4 standards. Primary and secondary JP-4 standards were prepared by dilution with HPLC grade, glass distilled carbon disulfide (Aldrich). Retention times and quantification of individual compounds was determined using dilutions of pure compounds. BTEX and alkanes were quantified by comparing the areas of unknowns with the areas of known standards. Analysis of headspace carbon dioxide, nitrogen, and oxygen were performed in two separate experiments using a Varian 3700 GC equipped with a Thermal Conductivity Detector (TCD). A molecular sieve (3 ft. x 1/8 inch, Supelco) was used to separate and quantify nitrogen and oxygen. Carbon dioxide was analyzed using an 80/100 Porapak Q column (12 ft. x 1/8 inch, Supelco). This column separated carbon dioxide from nitrogen and oxygen. Both analyses were performed at 40°C.

Gas chromatography-mass spectroscopy. Undiluted JP-4 was injected directly into a Varian 3400 Gas chromatograph equipped with a Petrocol DH (Supelco) 100 m x 0.25 mm capillary column (0.5 um film thickness). The temperature program used was 40°C for 10 min. followed by a temperature gradient from 40°C to 210°C at 3°C/min. An Incos 50 quadrupole mass spectrometer was used to detect and identify individual peaks. Scans were collected every 1.96 s. Tentative identification of individual compounds was by best match of the resulting spectral patterns with the National Bureau of Standards library.

Culture media Initial experiments were performed in 100 ml agitated cultures in 250 ml Erlenmeyer flasks. The phosphate ammonia magnesium medium (PAM) consisted of 2.22 g $K_2HPO_4{\cdot}3H_2O$, 7.26 g KH_2PO_4, 3.96 $(NH_4)_2SO_4$ and 0.2 g $MgSO_4.7H_2O$/liter, pH 7.1. For bioreactor studies, trace minerals were supplied by the tap water used to make the feedstock. Additionally, nitrogen and phosphorous were supplied to this system by adding 2 ml of a solution containing 851.6 mg NH_4HPO_4 and 2366.0 mg NH_4Cl per 3 liter of feedstock.

Results

Selection of source inocula. The four source inocula were screened alone and in combination for their ability to grow on JP-4 as a sole carbon source. Initially, all of the source inocula were grown on kerosine to ensure development of microorganisms capable of growing on the less water soluble components of JP-4. Following an acclimatization period, inocula were transferred to a culture medium containing JP-4 as the carbon source. Table 1 shows that all of these bacterial consortia utilized oxygen when JP-4 was the carbon source. Table 1 also shows that the rate of oxygen utilization was greatest in the cultures containing the bacterial consortium from the Los Angeles Air Force Base. Two ml

of this culture was transferred to a 250 ml Erlenmeyer flask containing 98 ml of PAM and 0.5 ml of JP-4 to maintain the culture and the remaining 98 ml of the original culture was used to inoculate the reactor. The remainder of the 100 ml of the soil slurry from which this culture had been isolated was also added to the reactor. An initial characterization of this consortium revealed the presence of *Pseudomonas luteola* and *Aeromonas sp.* Both of these bacteria were able to grow in Petri dishes on Noble agar containing PAM and JP-4 fumes as the sole carbon source. The soil from which this bacterial consortium was obtained was a sandy silt clay. An extensive analysis of this soil as recevied from the Los Angeles Air Force Base is presented in Table 2.

Reactor design. Figure 1 illustrates the design of the SBR used in this study. The SBR consisted of a 4 L kettle reactor, a 20 L feed tank, and a 4 L draw tank. The feed tank and the draw tank were both fitted with activated carbon traps so that components of JP-4 that may have been volatilized could be trapped and quantitated. A port on the kettle reactor was activated by a solenoid switch during the feed and draw cycle during which time atmosphere exchange occurred. The gas exchange port was also fitted with two activated carbon traps to sequester volatilized components of JP-4. Two Masterflex pumps were used in this system. The SBR was operated on a twelve hour cycle (Fill = 1 h, React = 9.5 h, Settle = 2 h and Draw 0.5 h). Automation was achieved using a Chrontrol timer. The low water level for the kettle reactor was 2 L and the high water level was 4 L. Thus 1 L of feedstock was supplied to the reactor each cycle. This reulted in a hydraulic retention time of 1.5 days. A schematic drawing illustrating the operation of an SBR is provided in figure 2.

Characterization of JP-4 and JP-4 in reactor feedstock. Figure 3 is a gas chromatogram of JP-4 received from Grissom Air Force Base. The chromatogram is consistent with the composition of JP-4 in that the concentration of C-15 and higher n-alkanes is limited to met a freezing point requirement below -72°C (3). Several non-purgeable and as yet unidentified amines were also found using GC-MS. Figure 4. is a gas chromatogram of the feed stock of water contaminated with JP-4 prior to addition to the SBR for treatment. The feedstock was made by adding 20 ml of JP-4 to 18 L of tap water followed by continuous mixing with a stir bar on a magnetic stirrer. By this method, concentrations of 30 - 39 mg/L of TPH were typically achieved. However, in comparing the feedstock (figure 4) with free product (figure 3), it is apparent that the proportion of BTEX is substantially greater in the feedstock than in the free product. This is because of the greater solubility of these compounds in water relative to the straight and branched chain alkanes.

Bioremediation of water contaminated with JP-4. The bioreactor illustrated in figure 1 was seeded, as described above, with a bacterial consortium obtained from the Los Angeles Air Force Base. The reactor was in continuous operation as a sequencing batch reactor for 135 days. Figure 5 compares a gas chromatogram of the feedstock with a gas chromatogram of the effluent following treatment in the SBR. In this experiment the TPH was reduced from 31.8 mg/L to 19 ug/L, a 99.94% reduction. Similarly, the benzene and toluene components of

JP-4 in this contaminated water were reduced from 7.5 mg/L and 11.2 mg/L to 1.2 ug/L and 0.6 ug/L, respectively, representing greater than 99.98% disappearance for both compounds. Analysis of activated carbon traps demonstrated that approximately 1% of the contaminating JP-4 was lost to volatilization. Although extensive bioremediation was achieved, components of JP-4 could still be detected in the treated water but only when the sample used for analysis was increased 100 fold. (Figure 6).

Oxygen consumption was also measured during some reactor cycles. In a typical run, 1.17 mg of oxygen were utilized per mg of TPH removed. These results correspond well with batch experiments conducted in serum bottles closed with Teflon lined butyl rubber stoppers. In these experiments, 1.12 mg of oxygen were utilized per mg of TPH removed. These results may be compared with theoretical results of 3.5 mg of oxygen utilized per mg TPH which is the maximum theoretical value that could be obtained if all of the TPH were converted to carbon dioxide and water and none to biomass.

Discussion

The purpose of this investigation was to develop an SBR capable of degrading constituents of JP-4 in contaminated to water to levels that are below regulatory concern. Additionally, it was our goal to design and operate the SBR in such a manner that loss due to air stripping would be minimized or negated.

Characterization of the contaminated water used in this investigation revealed that most (approximately 70 %) of the dissolved purgeable organics consisted of benzene, toluene, ethylbenzenes, and xylenes (i.e., BTEXs). This is most likely due to the greater water solubility of these JP-4 components relative to the alkanes and cycloalkanes which account for about 90% of the components found in JP-4. The actual distribution of JP-4 components found in ground water or surface water as a consequence of JP-4 spills is subject to a variety of phenomena including but not limited to the degree of weathering that occurs, the ambient temperature, and the vapor pressure and water solubility of individual components of JP-4. The type of soil, its ability to adsorb individual components of JP-4 and the degradative capabilities of the soil microorganisms will also effect the amount and composition of individual components found in contaminated ground water. In the present investigation, the artificially contaminated water used most probably mimics a spill in which the jet fuel is in direct contact with the ground water.

Results demonstrated that the contaminated water used in this investigation underwent extensive bioremediation and negligible air stripping during a 12 h cycle time in the SBR designed. Typically, the discharge water contained less than 20 ug/L of TPH and met the discharge levels required by all but the most stringent state regulatory agencies. For example, Alaska has a water quality standard of 15 ug/L for TPH. Research is in progress to develop systems to met even these stringent water quality standards. One promising design under investigation couples the use of biofilms and SBRs (e.g.,

Sequencing Batch Biofilm Reactors or SBBRs). Such systems are capable of reducing contaminants to less than 10 ug/L (4).

Acknowledgements

This research was supported by the Air Force Office of Scientific Research (AFOSR-91-0404). We thank Lt. Col. Harold Vice and Grissom Air Force Base for providing the samples of JP-4 used in this investigation.

Literature Cited

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3. Roberts, A.J. and Thomas, T.C. (1986) Characterization and Evaluation of JP-4, Jet A and Mixtures of these Fuels in Environmental Water Samples. Environ. Toxicol. Chem. 5:3-11.

4. Chozick, R. and Irvine, R.L. (1991) Preliminary Studies on the Granular Activated Carbon-Sequencing Batch Biofilm Reactor. Environ. Prog. 10:282-289.

Table 1. Oxygen Utilization by Several Bacterial Consortia in Cultures Containing JP-4 as a Carbon Source.*

Flask	Diesel Fuel Soil (gms of moist soil)	Gasoline Soil (ml of 10% slurry)	Municipal Sludge (ml)	Solmar Formulation (gm)	Oxygen Utilization (mg/20 h)
1	2.0	20.0	20.0	1.0	21
2	2.0	20.0	0	0	29
3	0	0	40.0	0	23
4	0	40.0	0	0	35
5	0	0	0	2.0	17
6	4.0	0	0	0	13

*Each culture was grown in 100 ml of PAM solution and 0.5 ml of Kerosine. After one week of incubation, 2 ml of each culture was transferred to closed flasks with gas sampling ports. Each flask contained 98 ml of PAM solution and 0.5 ml of JP-4. Samples of the headspace were removed periodically for determination of oxygen utilization. When necessary, headspace air was replenished. After five more days of acclimitization, the rate of Oxygen utilization over a twenty hour period was determined.

Table 2.

Characterization of a Gasoline Contaminated Soil from the Los Angeles Air Force Base (MacArthur Annex).

Physical Parameters

Hydraulic Conductivity	0.1 ft/day
Porosity	0.65
Sand	0.222
Silt	0.310
Clay	0.468
Fraction Dry Weight	0.617

Chemical Parameters

	mg/dry Kg soil
Total Petroleum Hydrocarbon	300 - 1,000[1]
	440[2]
Total Volatiles	129,000
COD	16,000
Total Phosphate	10.2
Total Nitrogen (Kjeldahl)	630
Cation Exchange Capacity	17 meq/100 gm
pH	7.2

Ionic Content

	mg/Kg
Anions	
NO_2^-	3
NO_3^-	20
Cl^-	700
SO_4^{2-}	1,247
Fl^-	100
PO_4^{3-}	5
Cations	
Na^+	450
NH_4^+	2
K^+	10
Ca^{2+}	2,100
Mg^{2+}	780
Fe^{3+}	22

[1]TPH assayed gravimetrically following Soxhlet extraction.
[2]TPH assayed by gas chromatography (FID) following heated purge and trap.

Figure Legends

Figure 1. Closed system Sequencing Batch Reactor Schematic.

Figure 2. Operation of a Sequencing Batch Reactor. The time intervals for each step correspond to those used in this investigation.

Figure 3. Gas chromatogram elution profile of JP-4.

Figure 4. Gas chromatogramof feedstock contaminated with JP-4.

Figure 5. Bioremediation of water contaminated with JP-4 in an SBR. Gas chromatograms of the feedstock prior to treatment and the effluent of an SBR following a twelve hour treatment in the SBR are compared.

Figure 6. Gas chromatogram of the effluent of an SBR following a twelve hour treatment in the SBR. This is the sample sample used in figure 5. However, a volume one-hundred fold greater was used to lower the detection limit.

Fig. 1

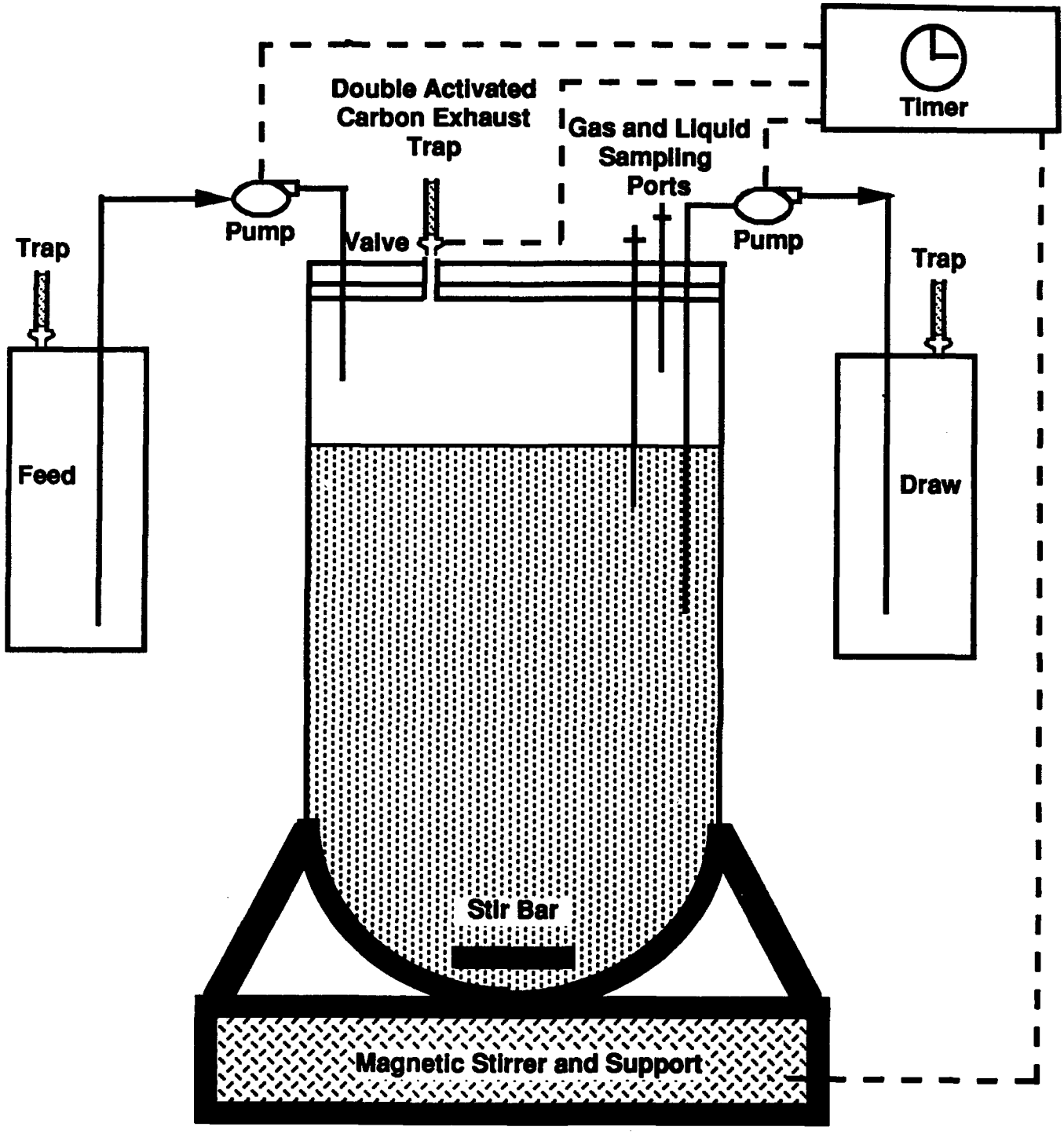
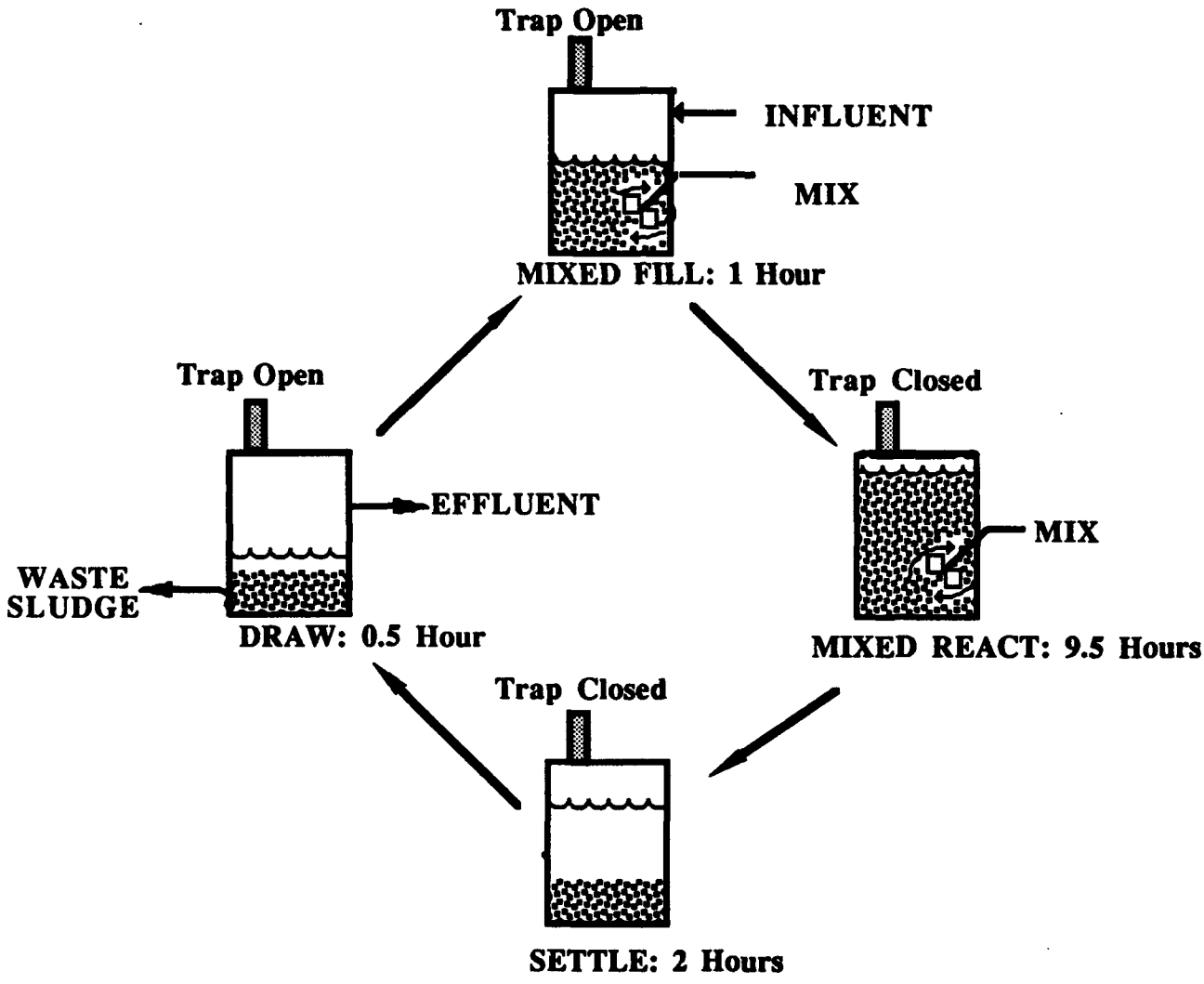
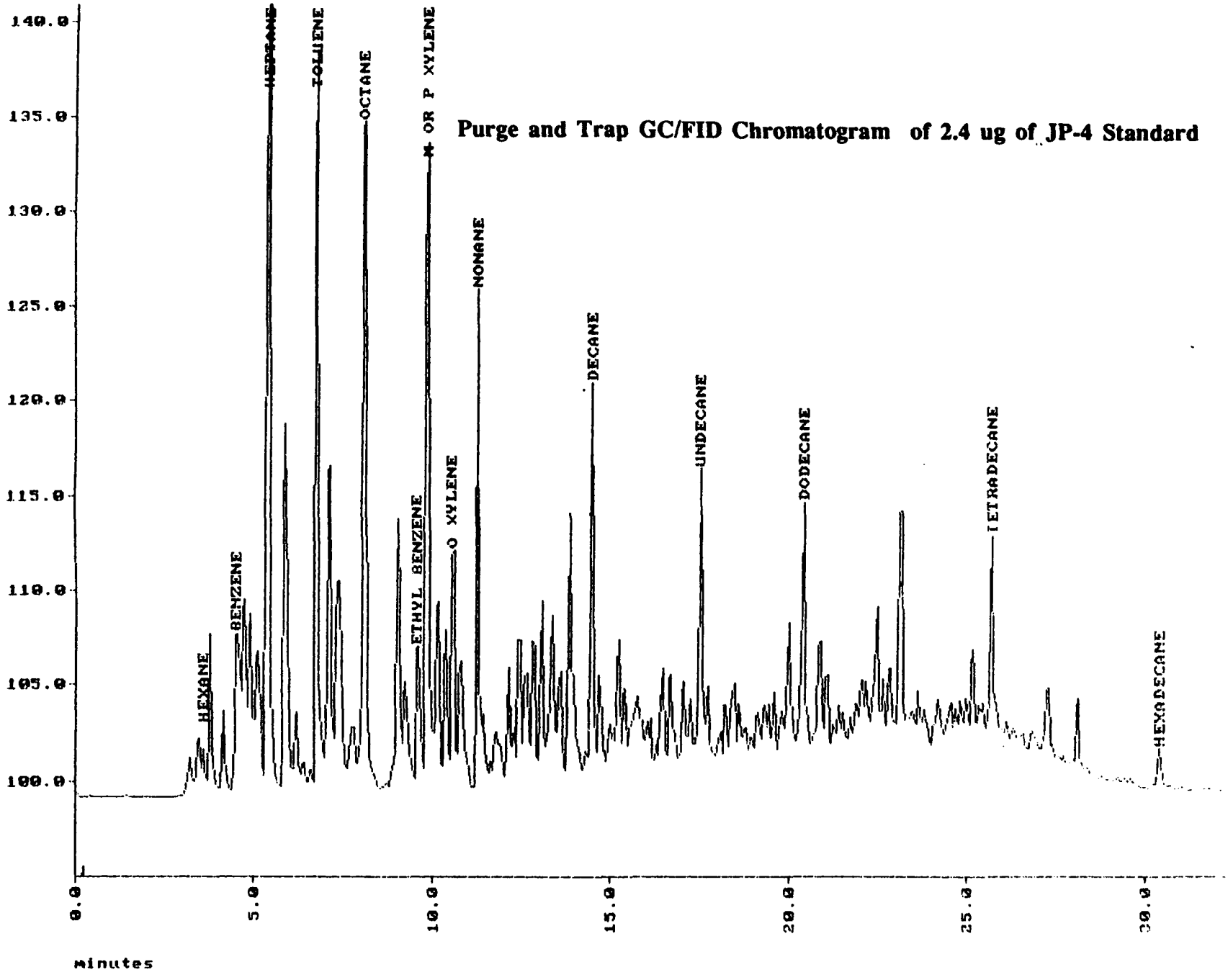


Figure 1. Closed System Sequencing Batch Reator Schematic.
note: reactor valve is only open during fill and draw.



SEQUENCING BATCH REACTOR (SBR) OPERATION FOR A TANK DURING ONE 12 HOUR CYCLE WITH FOUR TIME PERIODS (FILL, REACT, SETTLE, AND DRAW)

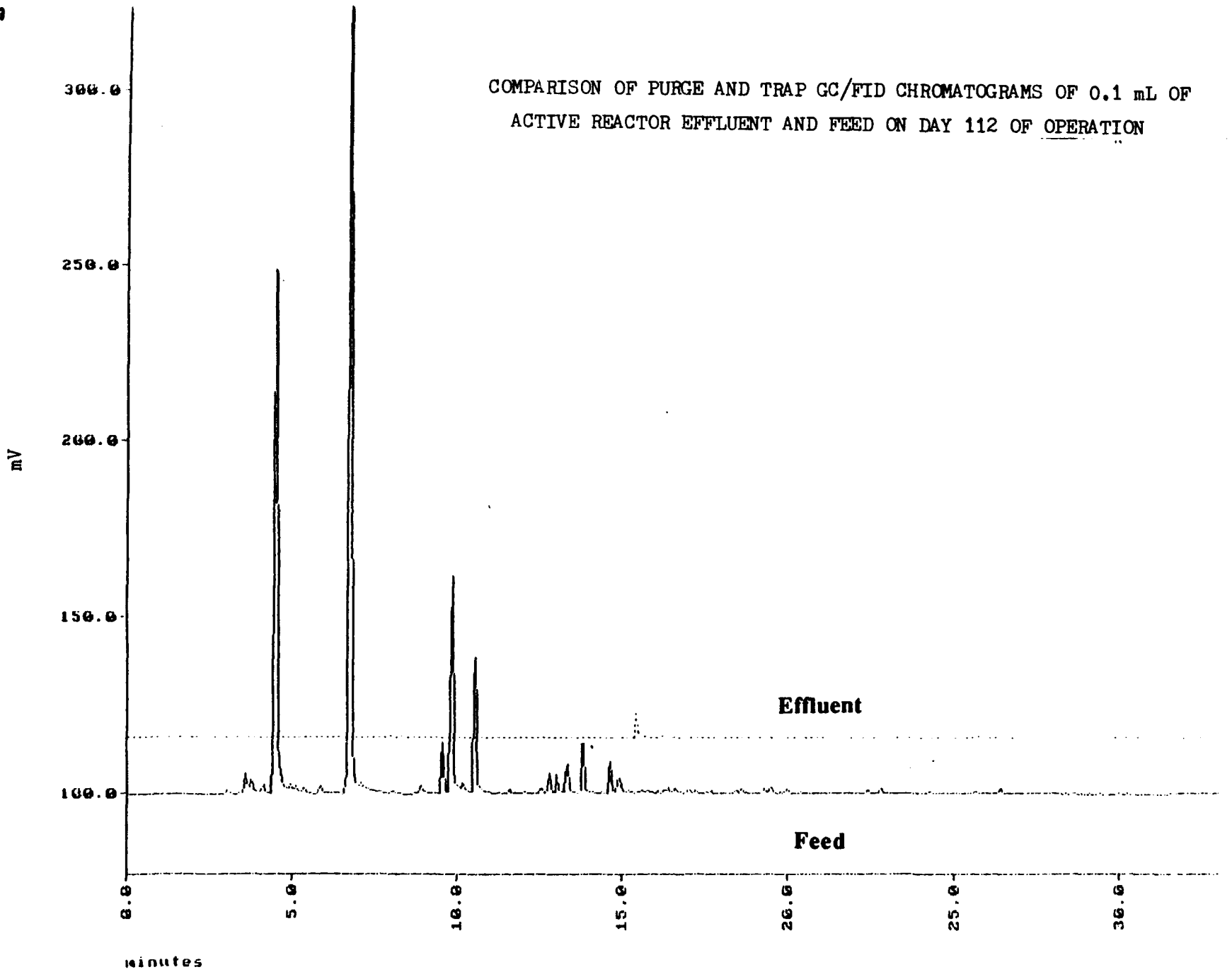
F.5.3


Purge and Trap GC/FID Chromatogram of 2.4 ug of JP-4 Standard
140.0
135.0
130.0
125.0
120.0
115.0
110.0
105.0
100.0
HEXANE
BENZENE
HEPTANE
TOLUENE
OCTANE
ETHYL BENZENE
M OR P XYLENE
O XYLENE
NONANE
DECANE
UNDECANE
DODECANE
TETRADECANE
HEXADECANE
0.0
5.0
10.0
15.0
20.0
25.0
30.0
minutes

Purge and Trap GC/FID Chromatogram of 0.1ml of Jp-4 Feed
HEXANE
BENZENE
HEPTANE
TOLUENE
OCTANE
ETHYL BENZENE
M OR P XYLENE
O XYLENE
NONANE
DECANE
UNDECANE
DODECANE
TETRADECANE
HEXADECANE
100.0
150.0
200.0
250.0
300.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
minutes

Fig 5

COMPARISON OF PURGE AND TRAP GC/FID CHROMATOGRAMS OF 0.1 mL OF ACTIVE REACTOR EFFLUENT AND FEED ON DAY 112 OF OPERATION



F.S. 4

PURGE AND TRAP GC/FID CHROMATOGRAM OF 10.0 mL OF ACTIVE REACTOR EFFLUENT ON DAY 112 OF OPERATION